

Comparison of Several Hot-Start Taq DNA Polymerases for Detection of Differentially Expressed Genes by GeneFishing

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Introduction

GeneFishing (Seegene) is a new proprietary method for detecting differentially expressed genes in two or more related samples. This two-step reverse transcription (RT)-PCR method modified from differential display PCR uses arbitrary primer pairs (annealing control primers, ACPs) at the PCR stage with a constant reverse primer (anchor ACP-T) which is also employed to prime the RT reaction. These ACPs have arbitrary 3'-end structures and constant 5'-end structures with an annealing regulatory region in between. With careful adjustment of cycling conditions, it is possible to allow only those cDNAs bearing complementary sequences to the variable 3' ends of the forward primers within a 5'-end region of approximately 2,000 nucleotides to be amplified.

The method is simple and straightforward to perform, but it would benefit from the use of a hot-start Taq DNA polymerase preventing undesired DNA synthesis during the reaction setup, especially when multiple samples are analyzed. Therefore, several commercially available hot-start Taq DNA polymerases were compared regarding their suitability for this purpose.

Materials and Methods

C1 and C2 cDNAs originating from gestation day 4.5 and 18.5 mouse fetuses, respectively, were used as templates (supplied in the GeneFishing kit). Four different hot-start enzymes were tested with the primer pair ACP-A1 and ACP-T. The reaction mixtures for PCR were prepared according to the manufacturer's recommendations using each enzyme's own buffer (Table 1). The reaction components were assembled on ice, then the tubes were transferred to a TGradient (Biometra) thermocycler. The Taq DNA polymerases were activated by incubating the tubes at 95°C for 5 minutes (hot-start

enzyme supplier A, FastStart) or 15 minutes (hot-start enzymes suppliers B and C). This initial denaturation/activation phase was followed by primer annealing at 50°C for 3 minutes and second-strand synthesis at 72°C

Table 1: PCR reaction mixtures (µl)

Polymerase	Supplier A	FastStart	Supplier B	Supplier C
dH ₂ O	28.5	34.6	33.75	30.75
10 x buffer	5	5 (with Mg)	5	5 (HP buffer)
25 mM MgCl ₂	5	1	2	5
10 mM dNTP	1	1	1	1
ACP-A1 primer	2	2	2	2
ACP-T primer	1	1	1	1
cDNA*	5	5	5	5
Taq polymerase	2.5	0.4	0.25	0.25
total (µl)	50	50	50	50

*approximately 8 ng/µl

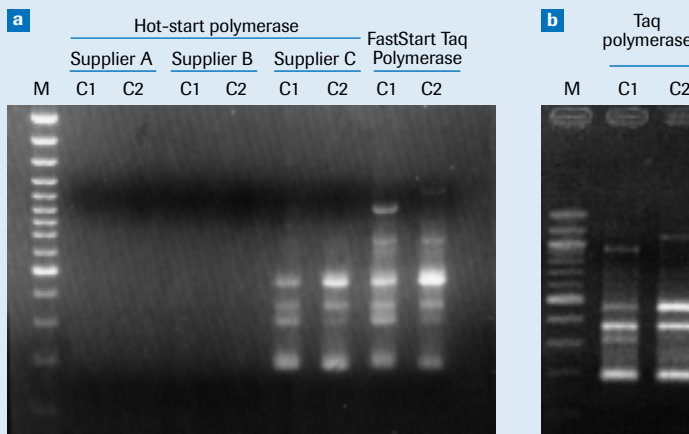
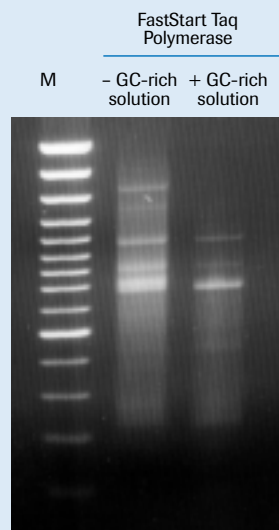


Figure 1: (a) Gel electrophoresis of PCR products generated with ACP-A1/ACP-T primers and with one of the indicated hot-start polymerases of suppliers A, B, C, or FastStart. The cDNA template was C1 or C2 (M, 100 bp marker). (b) The pattern of bands obtained with Taq polymerase from C1 and C2 cDNA templates with the same primer pair (M, 100 bp marker, Seegene).

Figure 2:
Banding patterns generated with FastStart Taq DNA Polymerase and ACP-A6/ACP-T primers from an untreated rat liver cDNA in the absence or presence of the GC-RICH Resolution Solution (M, 100 bp marker).



for 40 seconds. Then, a total of 40 cycles were run with the following program: 40 seconds at 94°C, 40 seconds at 65°C, and 40 seconds at 72°C. A final 5-minutes elongation at 72°C completed the cycling program.

Results and Discussion

The hot-start Taq DNA polymerases from suppliers A and B failed to amplify any product from the two cDNAs (Figure 1). The hot-start Taq DNA polymerase from supplier C generated the smaller products (up to 500 bp), but only FastStart was able to yield all the expected bands including the two largest ones (approximately 950 bp and 1,100 bp).

The degree of DNA polymerase activity is critical during the first cycle of the GeneFishing procedure, as second-strand synthesis is in process during this phase. All the remaining cycles have a relatively high annealing temperature. No product is generated if the first cycle is unsuccessful – in this case the arbitrary forward primer cannot anneal. Although all the hot-start enzymes tested were activated for the recommended periods at an appropriate temperature, the enzymes that failed may not have reached sufficient activity during this step. Alternatively, the buffer compositions of these enzymes did not favor annealing at the set temperatures.

Subsequent fine-tuning of the cycling conditions for FastStart Taq DNA Polymerase showed that even for this enzyme, it was advantageous to prolong the initial denaturation time from 5 minutes to 8 minutes to elevate its activity for the second-strand synthesis. Furthermore, the yield of long PCR products (up to 2 kb) improved when we expanded the elongation time to 2 minutes for

the first cycle, to 45 seconds for the next 15 cycles, and then stepwise by 1 second per cycle for the remaining 25 cycles.

FastStart Taq DNA Polymerase is supplied with an optional solution intended to enhance amplification of GC-rich templates. The effect of this solution was tested with another primer pair (ACP-A6/ACP-T) using rat liver cDNA as sample material. In the present experimental conditions, a reaction mixture without the GC-RICH Resolution Solution proved to perform better (Figure 2).

So far, we have employed almost 30 primer pairs in the GeneFishing procedure using FastStart Taq DNA Polymerase, and the results have invariably been highly satisfactory. The hot-start modification of the original method offers flexibility and convenience in a reaction setup without compromising the outcome. Thus, a large number of individual cDNA samples can be safely processed at the same time for comparative analysis of gene expression levels. ■

Product	Pack Size	Cat. No.
FastStart Taq DNA Polymerase	50 units	2 158 264
	100 units	2 032 902
	500 units	2 032 929
	4 x 250 units	2 032 937
	10 x 250 units	2 032 945
	20 x 250 units	2 032 953

